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We are entering a new era of medicine where genetic markers are going to be used to make clinical management decisions. My long term career goal is to further our understanding of the genetic alterations which characterize human breast cancer in a way that will eventually lead to early diagnosis, more effective treatment or prevention of the disease. The promise of research into breast cancer genetics is that it will provide us with new insight into the etiology of breast cancer that can be translated to strategies for early diagnosis and treatment for the larger population of women who develop breast cancer without having a genetic predisposition.

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## INTRODUCTION

As a physician-scientist, I have had extensive training in clinical oncology and in molecular biology and genetics; I am ideally positioned to bridge the gap between the two. The academic award has represented an outstanding opportunity for me to critically appraise the emerging role of genetics in clinical breast cancer care and forge new avenues of research. Toward this goal, I plan to accomplish the following during the period of my academic award.

1) perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.

2) develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.

3) develop a tissue repository composed of biological specimens from 500 patients with inherited breast cancer (e.g fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).

Using these unique resources, my future studies will characterize the molecular pathways that allow a normal breast cell to become cancerous in individuals who are genetically predisposed. I will also develop longitudinal follow up studies to correlate clinical outcomes with molecular characterization and epidemiologic risk factors. These studies will no doubt lead to an improved understanding of the biology of breast cancer, which will ultimately translate into more effective therapies.

## Task I

**Perform a thorough review of the cytogenetic and molecular genetics literature to identify potential chromosomal regions that may harbor genes whose abnormal function is critically involved in the development of breast cancer.**

This year we published three reviews, one book chapter, have one pending review, and one pending book chapter on the genetics of breast cancer. Thus we have completed Task I.

### Publications

Ademuyiwa FO, Olopade OI. Racial differences in genetic factors associated with breast cancer. Cancer Metastasis Rev. 22:47-53, 2003.

Olopade OI, Fackenthal JD, Dunston G, Tainsky MA, Collins F, Whitfield-Broome C. Breast Cancer Genetics in African Americans. Cancer 97:236-245, 2003.

Antman K, Abraido-Lanza AF, Blum D, Brownfield E, Cicatelli B, Debor MD, Emmons K, Fitzgibbon M, Gapstur SM, Gradishar W, Hiatt RA, Hubbell FA, Joe AK, Klassen AC, Lee NC, Linden HM, McMullin J, Mishra SI, Beuhaus C, Olopade FI, Walas K. Reducing disparities in breast cancer survival: a Columbia University and Avon Breast Cancer Research and Care Network Symposium. Breast Cancer Res Treat. 75(3): 269-80, 2002.

Principles of Molecular Oncology. Eds MH Bronchud, M Foote, G Giaccone, Olopade OI, P Workman. Humana Press.2003

Fackenthal,, JD and Olopade, OI (in press for *Endocrine-Related Cancer*). Cancer genetics of *BRCA1* and *BRCA2*.

Grushko T, Olopade OI. Genetic Changes in Breast tumors with hereditary Predisposition. Principles of Molecular Oncology Eds MH Bronchud, M Foote, G Giaccone, Olopade OI, P Workman. Humana Press (*in press*).

**Task II**

**Develop a robust panel of markers that can be used for clinical correlative studies of hereditary breast cancers.**

We have developed several probes for fluorescent *in situ* hybridization as well as optimized the conditions for several antibodies, which we have now applied to a well characterized panel of tumors in our tumor bank. Task II is completed and our findings are summarized below.

**a) Dissection of Cooperating Oncogenes involved in *BRCA1* tumor progression**

To examine whether amplification of *HER-2/neu* contributes to the aggressive biology of *BRCA1*-associated tumors, we performed fluorescence *in situ* hybridization (FISH) on formalin-fixed paraffin-embedded breast tumor tissue sections from 53 *BRCA1* mutation carriers and 41 randomly selected age-matched sporadic breast cancer cases. Although *BRCA1*-associated and sporadic tumors were equally likely (19% versus 22%) to exhibit *HER-2/neu* amplification (defined as a ratio of *HER-2/neu* copies to chromosome 17 centromere (*CEP17*) copies $\geq 2$), 6 (15%) of the sporadic tumors were highly amplified (defined as a ratio $\geq 5$) versus none of the *BRCA1*-associated tumors ($p = 0.048$). HER-2 protein overexpression as measured by immunohistochemical analysis (IHC) was not observed among the *BRCA1*-associated cases ($p = 0.042$). Four out of 21 (19%) sporadic tumors exhibited strong membranous staining of HER-2 (intensity level of 3+) as compared to 0/39 *BRCA1*-associated tumors. Our data suggest that a germ line mutation in the *BRCA1* tumor suppressor gene is associated with a significantly lower level of *HER-2/neu* amplification. Thus, it is possible that *BRCA1*- associated and *HER-2/neu*- highly amplified tumors progress through distinct molecular pathways and the aggressive pathologic features of *BRCA1*-associated tumors appear unrelated to amplification of the adjacent *HER-2/neu* oncogene (Grushko et al. 2002).

Using breast tumor tissues from a relatively large cohort of 102 women including 40 with deleterious *BRCA1* mutations and molecular cytogenetics techniques, we tested the hypothesis that the presence of a germ line *BRCA1* mutation might be associated with *MYC* amplification in somatic tumor cells. We observed a *MYC/CEP8* amplification ratio $\geq 2$ in 21 of 40 (53%) *BRCA1*-mutated compared to 14 of 62 (23%) sporadic tumors ($P=0.003$). Of interest, 8 (57%) of the 14 sporadic cases with *MYC* amplification were *BRCA1*-methylated. In total, *MYC* amplification was found in the majority of tumors with inactivated BRCA1 and at a higher frequency than in tumors without loss of BRCA1 (29/60, 48% versus 6/42, 14%; $p=0.0003$). Thus, our study confirms for the first time in a larger panel of tumors, data from DNA microarray studies that suggest that breast cancers arising in the setting of germ line *BRCA1* mutations have unique gene expression profiles, and sporadic tumors with methylated *BRCA1* may be misclassified with the *BRCA1*-mutation-positive group.

The importance of this work is to suggest that the aggressive features of *BRCA1*-associated tumors are in part due to MYC oncogenic activity and that MYC amplification contributes to tumor progression in both hereditary and sporadic BRCA1-deficient cells. This obviously has implications for understanding the molecular mechanisms for *de novo* gene amplification as well as for developing new drug targets. To our knowledge, this is the first comprehensive study of *MYC* gene amplification

in *BRCA1*-associated tumors using molecular-cytogenetic techniques. The manuscript has been accepted for publication in Clinical Cancer Research.

**b) . Hypermethylation of *BRCA1* and ER promoter**

We assessed *BRCA1* and estrogen receptor (ER) promoter methylation in 5 breast cancer cell lines and 132 primary breast tissues by Methylation-Specific (M-PCR). *BRCA1* and ER expression were determined in breast tumor cell lines and primary tissues by RT-PCR. In addition, we performed FISH using *BRCA1* and *CEP17* probes on both sporadic and *BRCA1*-associated hereditary breast cancer. We observed *BRCA1* methylation in the UACC-3199 positive control cell line and in 39 of 132 sporadic (29.5%) tumors. *BRCA1* methylation was correlated with chromosome 17 aneusomy and down-regulation or complete absence of the transcript. *BRCA1* methylation correlated inversely with age of onset: 40% of tumors from cases under 55 years old were methylated vs. 25% of cases over 55 years old (Table 4). The methylated cases were equally distributed among all histological types and there was no difference in the proportion of African American women (27.5%) vs. non-Hispanic White women with methylated tumors (28.6%). The majority of *BRCA1*-methylated cases (79%) were ER (-) and/or ER methylated. *MYC* and *HER2/neu* amplification in methylated tumors were intermediate in values between hereditary *BRCA1*-associated and sporadic unmethylated tumors, suggesting that *BRCA1* methylation might be incomplete in some tumors. These results suggest that silencing of the *BRCA1* gene by methylation occurs in a significant proportion of sporadic breast cancers and may be an early event during tumor progression (Wei et al. AACR 2003 abstract). There may be a slight difference in the proportion of black women with methylated tumors.

**Publications**

Tatyana A. Grushko, James J. Dignam, Soma Das, Anne Blackwood, Charles M. Perou, , April J. Adams, Fitsum G. Hagos, Lise Sveen , Karin K. Ridderstråle, Kristin Anderson, Barbara L. Weber Olufunmilayo I. Olopade, M.D. *MYC* is amplified in *BRCA1*-associated breast cancers. Manuscript in press. Clinical Cancer Research.

Min-Jie Wei, Tatyana Grushko, Soma Das, James Dignam, Fitsum Hagos, Lise Sveen, James Fackenthal and Olufunmilayo I Olopade. Methylation of the *BRCA1* Promoter in Sporadic Breast Cancer Related to *BRCA1* Copy Number and Pathologic Features. Manuscript in preparation.

Wei M-J, Grushko T, Das S, Dignam J, Hagos F, Sveen L, Fackenthal J and Olopade OI. Methylation of the *BRCA1* Promoter in Sporadic Breast Cancer Related to *BRCA1* Copy Number and Pathologic Features. American Association for Cancer Research, 94th Annual Meeting (poster with published abstract), 2003.

**Task III**

**Develop a tissue repository composed of biological specimens from 500 patients with familial or hereditary breast cancer (e.g fresh frozen tumor specimens, or paraffin embedded tumor specimens and normal blood lymphocytes, DNA and sera whenever possible).**

After several delayed attempts to get approval for a clinical protocol for the tumor bank from the army despite approval from our local IRB, we have enrolled 25 patients. Due to low accrual despite the one year no cost extension provided to us last year, we wish to terminate the protocol as it is no longer viable.

## KEY RESEARCH ACCOMPLISHMENTS:

We are defining important pathways in inherited BRCA1-associated breast cancer tumor progression.

## REPORTABLE OUTCOMES:

Based on this academic award from the Army, I am now a full-fledged breast cancer researcher. This work has led to two RO1s funded by the National Institutes of Health.

Academic Productivity in 2003.

Olopade OI, Fackenthal JD, Dunston G, Tainsky MA, Collins F, Whitfield-Broome C. Breast cancer genetics in African Americans. Cancer. 2003 Jan 1;97(1 Suppl):236-45.

Wei M-J, Grushko T, Das S, Dignam J, Hagos F, Sveen L, Fackenthal J and Olopade OI. Methylation of the *BRCA1* Promoter in Sporadic Breast Cancer Related to *BRCA1* Copy Number and Pathologic Features. American Association for Cancer Research, 94th Annual Meeting (poster with published abstract), 2003

Nanda, R., Schumm, P., Cummings, S., Fackenthal, J., Hagos, F., Esserman, L., Neuhausen, S., Olopade, OI. *BRCA1* and *BRCA2* mutations in an ethnically diverse population of high-risk individuals: comparisons between African American and Caucasian families. American Society of Clinical Oncology Annual Meeting for 2003 (poster discussion with published abstract).

## CONCLUSIONS:

The observed similarities in molecular pathogenesis between *BRCA1*-mutated and *BRCA1*-methylated tumors led us to propose a tumor progression model in which early loss of *BRCA1* causes defects in chromosome structure, cell division, and viability, so that a *BRCA1*-deficient cell must acquire additional alterations that overcome these problems and presumably force tumor evolution down a limited set of pathways. Our FISH results are consistent with data from DNA microarray studies that suggest that breast cancers arising in the setting of germ line *BRCA1* mutations have unique gene expression profiles, and sporadic tumors with methylated *BRCA1* may be misclassified with the *BRCA1*-mutation-positive group. A review of the set of genes published by Heldenfalk et al. (Hedenfalk et al. 2001) and in the paper by van 't Veer et al. (van 't Veer et al. 2002) demonstrated that *MYC* on 8q was overexpressed in *BRCA1* mutation carriers. In addition, the *BRCA1* mutant tumors we have studied appear to have a profile that is most consistent with the basal-like subtype suggested by Perou et al. (Perou et al. 2000), based on the following observations. First, both (meaning sporadic basal-like tumors and *BRCA1* mutant tumors) tend to be high grade, ER/PR negative and *HER2/neu*-negative, and both show the high expression and/or amplification of *MYC*. In fact, *MYC* emerged as one of the most relevant genes that defined the basal-like group and was expressed more than 2-4 fold above background in the majority of cases (Perou, unpublished results). Moreover, we have shown that *BRCA1*-mutated tumors express specific basal cytokeratins in a manner suggestive of an ER-negative basal-like epithelial cell of origin (Olopade and Grushko 2001) and are never associated with high levels of *HER-2/neu* amplification (Grushko et al. 2002). Therefore, it is reasonable to suggest that *BRCA1*-mutated tumors are mostly basal-like (ER-, HER2-) and that *MYC* amplification/overexpression further defines *BRCA1*-deficient tumor cells.

**REFERENCES:**

van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH.Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002 Jan 31;415(6871):530-6.

Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi OP, Wilfond B, Borg A, Trent J. Gene-expression profiles in hereditary breast cancer. N Engl J Med. 2001 Feb 22;344(8):539-48.

Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumors. Nature. 2000 Aug 17;406(6797):747-52.

**APPENDICES:**

N/A